PEJABAT PENGURUSAN & KREATIVITI PENYELIDIKAN
RESEARCH CREATIVITY AND MANAGEMENT OFFICE [RCMO]

LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*
FINAL REPORT OF SHORT TERM RESEARCH PROJECTS

1) **Nama Ketua Penyelidik :**
Name of Research Leader :

Ketua Penyelidik *Research Leader*	PTJ *School/Centre*
Dr. Widowati Witjaksono	School of Dental Sciences

Nama Penyelidik Bersama
(Jika berkaitan) **:**
Name/s of Co-Researcher/s
(if applicable)

Penyelidik Bersama *Co-Researcher*	PTJ *School/Centre*
Prof. H. AB. Rani Samsudin	School of Dental Sciences
Dr. Lin Naing @ Moh.Ayub Sadiq	School of Dental Sciences
Dr. Mon Mon Tin Oo	School of Dental Sciences
Dr. Ema Mulyawati	Change affiliation / institution in Indonesia

2) **Tajuk Projek :**

Sealing Ability of Hydroxyapatite as a Root Canal Sealer: *In vitro* Study

3) **Abstrak untuk penyelidikan anda**

(Perlu disediakan di antara 100 – 200 perkataan di dalam **Bahasa Malaysia dan Bahasa Inggeris.** Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & luar).

Abstract of Research

(Must be prepared in 100 – 200 words in Bahasa Malaysia as well as in English. This abstract will later be included in the Annual Report of the Research and Innovation Section as a means of presenting the project findings of the researcher/s to the university and the outside community)

English

Obturation in root canal treatment consists of placing an inert filling material in the space previously occupied by pulp tissue.Gutta-percha is used with various techniques for obturation of the root canal system, and if combine with lateral condensation remain the most widely accepted and used obturation technique.The most common cause of failure involving endodontic treatment can be attributed to the lack of an apical seal leading to leakage at the apex. Hydroxyapatite (HA) is the most thermodynamically synthetic calcium phosphate ceramic, and has indicated useful as a sealer because can seal a furcation perforation, is shown to be biocompatible and also has potential to promote the healing of bone in endodontic therapy. The objective of this study is to determine the sealing ability of HA produced by School of Engineering, Universiti Sains Malaysia (USM) when used as a sealer in root canal obturation, compare with Tubli-seal (Zinc-Oxide base) and Sealapax (Calcium Hydroxyde base) sealers.

Forty five single rooted human extracted anterior teeth were instrumented and randomly divided into three experimental groups of 15 teeth each. All teeth in the experimental groups were obturated with laterally condensed gutta percha technique. Teeth in the first group were sealed using Zinc-Oxide (ZnO) based sealer and those of second group using Calcium Hydroxide (CaOH) based root canal sealer. Third experimental group was sealed using HA from School of Engineering,USM. Teeth were then suspended in 2% methylene blue. After this, teeth were demineralized dehydrated and cleared. Linear dye penetration was determined under magnifying lense with calibrated eye piece.

Statistical analyses of the linear dye penetration were performed with Kruskal Wallis test. As for the inter group comparison between HA and ZnO groups and between HA and CaOH groups were analysed by Mann-Whitney test.

The results showed that dye penetration for group which were sealed with HA exhibited the lowest penetration and it showed that there was a statistically significant difference both between HA and ZnO groups and also between HA and CaOH groups ($p<0.001$).

We conclude that value added HA based endodontic material which were produced by USM can be used as a root canal sealing materials when it used in combination with epoxy resin since it leaked comparatively less as compared to ZnO and CaOH sealers. Before reaching a definitive conclusion, this material requires further extensive exploration both clinically and *in vitro* .

Bahasa Malaysia.....

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Pengisian didalam perawatan saluran akar bermaksud menempatkan materi yang mudah dibentuk kedalam rongga ataupun ruang yang semula terisi oleh jaringan pulpa. Untuk keperluan tersebut biasanya digunakan gutta-percha dengan bermacam teknik untuk mengisi sistim saluran akar, dan apabila dikombinasi dengan kondensasi lateral maka ianya merupakan teknik yang paling banyak serta luas digunakan oleh para pakar pergigian. Kes kegagalan yang paling banyak terjadi didalam perawatan endodontik dapat disebabkan oleh kehilangan daya rekat di sekitar apikal gigi yang menyebabkan terjadinya kebocoran di apikal gigi. Hydroxyapatite (HA) adalah calcium phosphate ceramic sintetis yang paling bersifat termodinamis dan berindikasi untuk digunakan sebagai perekat karena dapat menutup lubang furkasi, memperlihatkan biokompatibilitas dan juga mempunyai potensi untuk mempromosi proses kesembuhan tulang pada terapi endodontik.

Tujuan dari penyelidikan ini adalah untuk menentukan kemampuan daya rekat HA produk dari Pusat Pengajian Kejuruteraan Universiti Sains Malaysia (USM) apabila digunakan sebagai perekat dalam proses pengisian saluran akar, serta dibandingkan dengan perekat perekat lain (sedia ada di pasaran) seperti Tubli-seal (berazas Zinc-Oxide) dan Sealapex (berazas Calcium Hydroxide).

Empat puluh lima batang gigi anterior berakar tunggal dari pasien yang telah sedia dicabut dilakukan instrumentasi, seterusnya dibagi secara rambang kepada 3 grup yang masing masing berisi 15 batang gigi. Kesemua gigi dalam grup eksperimen diisi gutta-percha dengan teknik lateral kondensasi. Gigi gigi dalam kelompok pertama ditutup dengan perekat berazas Zinc-Oxide (ZnO) dan gigi gigi dalam kelompok ke dua ditutup dengan perekat berazas Calcium-Hydroxide (CaOH). Kelompok eksperimen ke tiga ditutup dengan HA produk dari Pusat Pengajian Kejuruteraan,USM. Kesemua gigi dalam kelompok eksperimen disuspensikan dalam 2 % methylen blue secara berasingan. Kemudian gigi gigi tersebut dilakukan demineralisasi, dehidrasi dan penjernihan. Penetrasi pewarna secara linier ditentukan dengan bantuan lensa pembesar dengan pengamatan yang telah dikalibrasi.
Analisa statistik dari penetrasi warna secara linier dilaksanakan dengan test "Kruskal Wallis". Sedangkan perbandingan antara grup HA dengan grup ZnO dan grup HA dengan grup CaOH dianalisa dengan test " Mann-Whitney"
Hasil penyelidikan menunjukkan bahawa penetrasi warna yang terendah ada pada kelompok HA, dan secara statistik ada perbezaan yang bermakna diantara grup HA dengan grup ZnO, serta juga ada perbezaan bermakna diantara grup HA dan grup CaOH (p< o.oo1).
Kesimpulannya, HA yang dicampuri dengan material berharga produk dari USM dapat pula digunakan sebagai materi penutup saluran akar apabila ianya digunakan bersama dengan resin epoxy, karena mempunyai kadar kebocoran yang rendah bila dibandingkan dengan grup ZnO dan grup CaOH. Sebelum mencapai kesimpulan akhir, maka perlu dilakukan investigasi yang lebih jauh dan luas pada materi ini baik secara klinikal maupun *in vitro*.

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4) Sila sediakan Laporan teknikal lengkap yang menerangkan keseluruhan projek ini.
[Sila gunakan kertas berasingan]
Kindly prepare a comprehensive technical report explaining the project
(Prepare report separately as attachment) (attached here with)

Senaraikan Kata Kunci yang boleh menggambarkan penyelidikan anda :
List a glosssary that explains or reflects your research:

	Bahasa Malaysia	Bahasa Inggeris
Penutup Pengisi ,	daerah apical, Perekat endodontik, Lensa pembesar	Apical seal, Endodontic sealers, Obturant, Magnifying lense

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5) **Output Dan Faedah Projek**
Output and Benefits of Project

(a) * Penerbitan *(termasuk laporan/kertas seminar)*
Publications (including reports/seminar papers)
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).
(Kindly state each type, title, author/editor, publication year and journal/s containing publication)

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This study will be presented on the International Medical and Health Congress In Kota Bharu - Kelantan on 25 - 26 May 2007, and the title is: Sealing Ability of Hydroxyapatite as a Root Canal Sealer: *In vitro* study
.....
Author: Widowati Witjaksono, Lin Naing, Ema Mulyawati, AR.Samsudin, Mon Mon Tin Oo
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After presentation, the next step must be sent to the international or semi international journal, we prefer to send in MJMS (The Malaysian Journal of Medical Sciences) published by the School of Medical Sciences Universiti Sains Malaysia since the material used is made by USM, but if they take too long time to let us know whether they accept or refuse, then
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Send to
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Majalah Kedokteran Gigi (Dental Journal), published by School of Dentistry Airlangga University in Surabaya, Indonesia (usually they take an average time of 2 weeks to reply accept or refuse), then we will make any corrections they suggest untill it can be published, usually will take an average time for 3 - 4 months /depend on corrections made
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(b) **Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten atau Impak kepada dasar dan masyakarat.**
Other benefits such as product development, product commercialisation/patent registration or impact on source and society

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USM can proceed to **Hydroxyapatite based USM's sealer**, off course it requires further extensive exploration, especially under the field of Dental Material
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* Sila berikan salinan

* *Kindly provide copies*

(c) **Latihan Gunatenaga Manusia**
Training in Human Resources

i) Pelajar,Siswazah : .....
Postgraduate students:
(perincikan nama, ijazah dan status)
(Provide names, degrees and status)
.....
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ii) Pelajar Prasiswazah : .....
Undergraduate students:
(Nyatakan bilangan)
(Provide number)
.....
1 (one) student, Name: Sylvester Peter Nansi, matrix no. 70716
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iii) Lain-Lain : .....
Others:
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6. **Peralatan Yang Telah Dibeli :**
Equipment that has been purchased:

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Computer Laptop: Hewlet Packard / Compaq DV 3109
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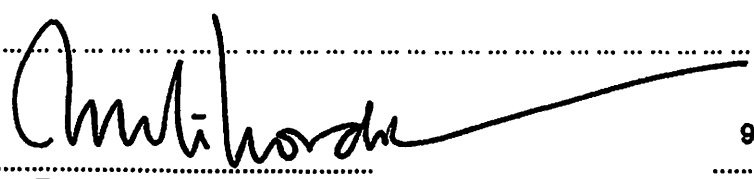
KOMEN JAWATANKUASA PENYELIDIKAN PUSAT PENGAJIAN
Comments of the Research Committees of Schools/Centres

Laporan Memuaskan.



9 April 2007

TANDATANGAN PENGERUSI
JAWATANKUASA PENYELIDIKAN PUSAT PENGAJIAN
Signature of Chairman
[Research Committee of School/Centre]

TARIKH
Date

PROF. DR. RUSLI BIN NORDIN
Profesor Perubatan Masyarakat
Timbalan Dekan
(Penyelidikan & Pengajian Siswazah)
Pusat Pengajian Sains Pergigian
USM Kampus Kesihatan
16150 Kubang Kerian
Kelantan

Updated : 16MAC2006

UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN WANG PENYELIDIKAN GERAN USM(304)
PENYATA PERBELANJAAN SEHINGGA 31 MAC 2007

Jumlah Geran:	RM	Tiada Rekod	Ketua Projek:	DR. WIDOWATI WITJAKSONO
Peruntukan 2005 (Tahun 1)	RM	3,935.00	Tajuk Projek:	Sealing Ability of Hydroxy apatite as a Root Canal Sealer: In Vitro Study
Peruntukan 2006 (Tahun 2)	RM	3,935.00		
Peruntukan 2007 (Tahun 3)	RM	0.00	Tempoh:	1 Apr 05-31 Mac 07
			No.Akaun:	304/PPSG/6131361

Kwg	Akaun	PTJ	Projek	Donor	Peruntukan Projek	Perbelanjaan T'kumpul Hingga Tahun Lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
304	11000	PPSG	6131361		-	-	-	-	-	-	-
304	14000	PPSG	6131361		-	-	-	-	-	-	-
304	15000	PPSG	6131361		-	-	-	-	-	-	-
304	21000	PPSG	6131361		-	-	-	-	-	-	-
304	22000	PPSG	6131361		-	-	-	-	-	-	-
304	23000	PPSG	6131361		-	-	-	-	-	-	-
304	24000	PPSG	6131361		-	-	-	-	-	-	-
304	25000	PPSG	6131361		-	-	-	-	-	-	-
304	26000	PPSG	6131361		-	-	-	-	-	-	-
304	27000	PPSG	6131361		7,870.00	3,575.00	4,295.00	-	-	-	4,295.00
304	28000	PPSG	6131361		-	-	-	-	-	-	-
304	29000	PPSG	6131361		-	-	-	-	250.00	250.00	(250.00)
304	32000	PPSG	6131361		-	-	-	-	-	-	-
304	35000	PPSG	6131361		-	-	-	3,399.00	-	3,399.00	(3,399.00)
					7,870.00	3,575.00	4,295.00	3,399.00	250.00	3,649.00	646.00

UNIVERSITI SAINS MALAYSIA

LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*
FINAL REPORT OF SHORT TERM RESEARCH PROJECTS

Sealing Ability of Hydroxyapatite As A Root Canal Sealer: *In Vitro* Study

Authors: Widowati Witjaksono, Lin Naing, Ema Mulyawati, AR.Samsudin, Mon Mon Tin Oo

Table 2. Comparison of dye penetration (in mm) between study groups

Group	*n*	Dye Penetration		X^2 Stat. (*df*)	*P* value[a]
		Median (IQR)	Min.-Max.		
Zinc Oxide	15	2.0 (0.5)	1-3	31.00 (2)	<0.001
Calcium Hydroxide	15	2.0 (1.0)	1-3		
Hydroxyapatite	15	0.0 (0.5)	0-1		

[a] Kruskal Wallis test
IQR=Interquartile range; Min.=Minimum; Max.=Maximum;

Table 3 Comparison of dye penetration between Hydroxyapatite (HA) and others

Comparison	*Z* Stat.	*P* value[a]
Zinc Oxide vs HA	-4.76	<0.001
Calcium Hydroxide vs HA	-4.77	<0.001

[a] Mann-Whitney test; *P* value is adjusted using Bonferroni procedure for multiple comparison

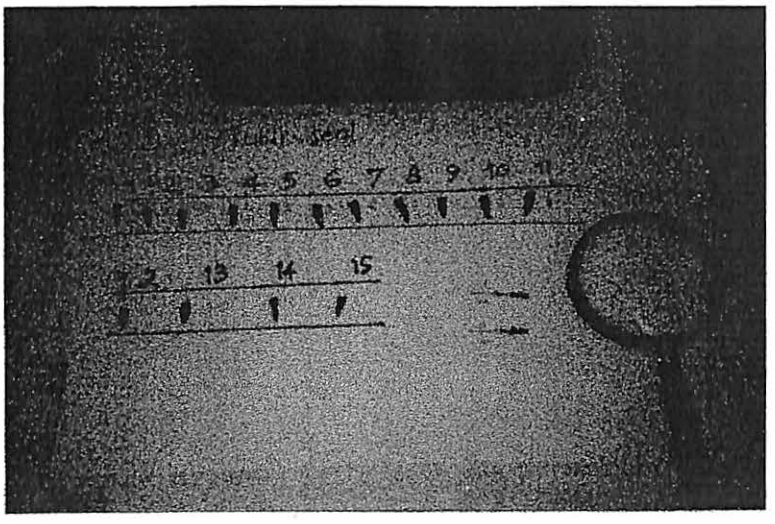


Fig1.Measurement of apical leakage in Tubli seal (ZnO base)

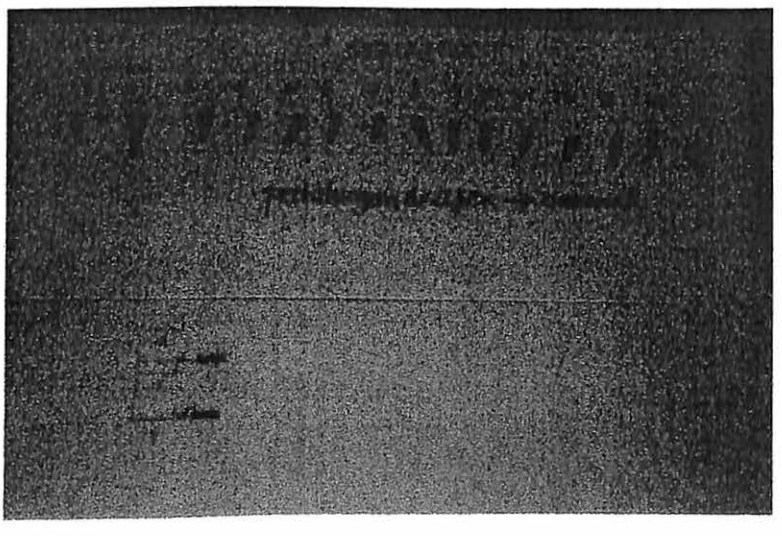


Fig2. Measurement of apical leakage in Sealapex (CaOH base)

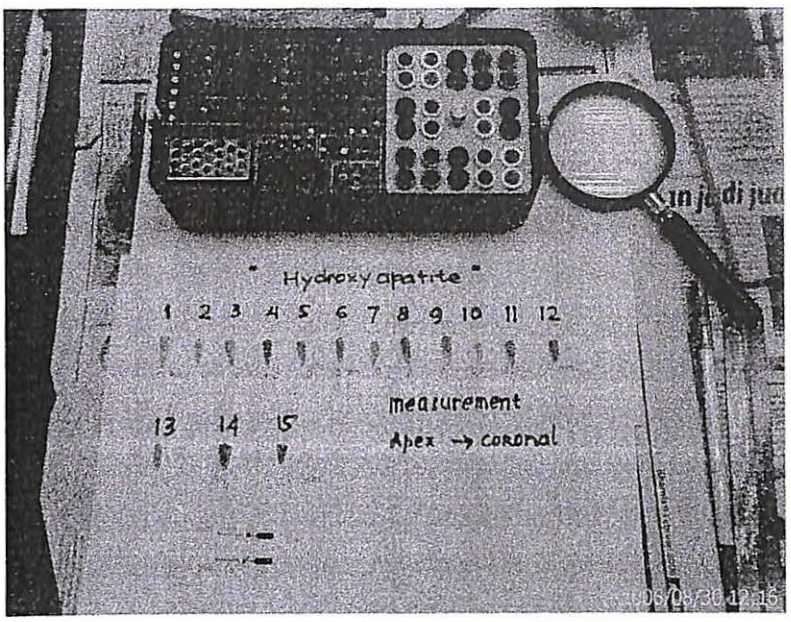


Fig3. Measurement of apical leakage in Hydroxyapatite

In the present study, measurements of maximum linear dye penetration were made to quantify the relative leakage (Figs 1,2,3). Dye penetration data for all the three groups are summarized in Table 1.
In group C teeth which were filled with laterally-condensed gutta-percha and hydroxyapatite (HA) sealer exhibited the lowest minimum-maximum (min-max) value of dye penetration. The min-max of dye penetration for group C(HA) was between 0-1mm. The min-max of dye penetration for group A teeth which were filled with laterally-condensed gutta-percha and zinc oxide (ZnO) base sealer was between 1-3 mm. The corresponding values for group B teeth which were filled with laterally condensed gutta-percha and calcium hydroxide (CaOH) base sealer was also between 1-3 mm. Ten samples in group C (HA) showed no dye penetration whereas all samples in group A and B showed dye penetration. The comparison of the three study groups using Kruskal Wallis test (table 2) revealed that there were at least one significant difference among the study groups ($p<0.001$).
Further Mann-Whitney test (post-hoe multiple comparisons between two groups) (table3) revealed that HA group has significancy lesser penetration compared to ZnO or CaOH groups ($p<0.001$)

DISCUSSION

HA is one of the ceramic materials commercially used for orthopaedic and dental implants, and it forms the principal mineral component of bone and comprises 60 % to 70 % of the calcified skeleton. It's chemical composition is $Ca_{10}(PO_4)_6(OH)_2$ and it has been produced synthetically since the early 1970's and used clinically for the last 20 years.[12,13] It has received considerable attention over the past two decades primarily because of it's excellent biocompatibility with hard tissues.[14,15] and when placed in contact

with viable bone, result in osteoconduction and osteointegration.[16,17] HA does not cause a chronic inflammatory respons, toxic reactions or a foreign body giant cell reaction.[13] Although HA is a promising implant material, the greatest stumbling block to it's wider application and utilization is the brittleness of the material and it's low strength for load-bearing applications.[18] Thus the material used in this study is the value added HA based material which were produced by a group of scientist at the School of Engineering Universiti Sains Malaysia (USM) in granules form. The pure HA has been added with zirconia and other additional components, hot pressed and then sintered at a temperature of 1300°C, to increase the toughness and strength of HA ceramic. Composites formed by HA ceramic in combination with zirconia have been proven not to produce any local or systemic adverse reactions or any cytotoxix effects in various *in vivo* studies.[19] This material showed no decrease in strength after ageing up to 1 year, which is in agreement with the study done by Shimizu et.al in 1993.[20]

It has been recognized for decades that the ideal end result of root canal therapy would be a closure of the apical foramen with reparative cementum. The goals for stability of successful endodontic therapy are total obliteration of the canal and perfect sealing of the apical foramen at the dentino-cemental junction and accessory canals at locations other than the root apex with an inert, dimensionally stable and biologically compatible material.[21] According to Timpawat et.al,[22] endodontic sealers are used to eliminate the interface between the gutta-percha and the dentinal walls. Thus, the quality of the filling depends largely on the sealing capacity offered by sealers.[23,24]

From this study an average leakage values of Zn O base sealer and CaOH base sealer both were minimum of 1 mm to maximum of 3 mm. The lesser value of dye penetration shown by value added HA sealer in the present study may be because of the better sealing abilities of HA. One possible explanation for this observed difference may be that HA has ability to bind strongly with natural bone tissue[25],and synthetic HA has the same chemical composition as biological HA and thus mimics many properties of natural bone.[26]As for epoxy-resin based endodontic sealers to the human dentin showed a higher capacity to attach to the dentinal walls than other endodontic sealers and provide bonding between it and gutta-percha points.[27]However the exact mechanism by which HA is incorporation with epoxy resin, then can be function as a good root canal sealer in the present study remain far from clear. It would be necessary to carry out further studies in order to make a larger evaluation of these value added HA based endodontic materials as well as their potential benefits. The *in vivo* evaluation should be done to assess the reaction to this value added HA as compared to the pure HA.

It can be concluded from this study that the value added HA based endodontic material which were produced by School of Engineering USM leaked comparatively less as compared to Zn O and CaOH sealer when it used in combination with epoxy resin.

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Subject: RE: Pemberitahuan biaya administrasi penerbitan artikel MKG
From: "widowati wat jaksono" <widowati@kb.usm.my>
Date: Fri, 20 Apr 2007 11:23:39 +0800
To: "'Majalah Kedokteran Gigi'" <dental_journal@yahoo.com>
CC: "'Abdul Hakim'" <abdhakim@kck.usm.my>

Chief Editor
Majalah Kedokteran Gigi (Dental Journal)
Dr.R. Darmawan Seitjanto, drg, M.Kes
Faculty of Dentistry, Airlangga University

Re: Manuscript submission

Dear Doctor,

Pertama-tama kami ucapkan terimakaseh atas informasi administrasi yang dikirimkan pada tarikh 15 April'06 yang lalu. Pada kesempatan ini pula kami ucapkan " Tahniah"diatas segala kerjaya puan dan tuan didalam memajukan Majalah Kedoktoran Gigi (Dental Journal)hingga dikenali ramai baik dalam skala nasional maupun internasional, terutama melalui website yang ada.
Kami dari negeri serumpun, memang berminat untuk mengirimkan naskah kepada majalah yang tuan kelola. Walaubagaimanapun kami mohon ijin terlebih dahulu untuk menyambung email kami ini dalam bahasa Inggris, agar mudah dimengerti oleh para author yang lain.

By this mail I would like to attach our article herewith with the title: "Sealing Ability of Hydroxyapatite As A Root Canal Sealer: In vitro Study" to Dental Journal. This article is not yet in a process on any journals or other institutions for publications.
I am happy if you let me know ASAP whether you'll accept or not this manuscript to review and publish in your journal
I am looking forward to hear from you soon,
Thanking you in advance,

Best Regard,

Widowati Witjaksono
(First Author)
Note: C/c for En.A.Hakim (R&D PPSG) and authors

From: Majalah Kedokteran Gigi [mailto:dental_journal@yahoo.com]
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Dengan hormat,

Sehubungan dengan permohonan saudara perihal informasi biaya administrasi pemuatan artike Majalah Kedokteran Gigi (*Dental Journal*), maka dengan ini diberitahukan bahwa menurut ketentuar yang berlaku setelah artikel dimuat oleh Majalah Kedokteran Gigi (*Dental Journal*) penulis akar dikenai biaya administrasi sebagai berikut:

Sealing Ability of Hydroxyapatite as A Root Canal Sealer: *In vitro* study

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ABSTRACT

Obturation in root canal treatment consists of placing an inert filling material in the space previously occupied by pulp tissue.Gutta-percha is used with various techniques for obturation of the root canal system. Hydroxyapatite (HA) is the most thermodynamically synthetic calcium phosphate ceramic, and has indicated useful as a sealer because can seal a furcation perforation, is shown to be biocompatible and also has potential to promote the healing of bone in endodontic therapy. The objective of this study is to determine the sealing ability of HA produced by School of Engineering, Universiti Sains Malaysia (USM) when used as a sealer in root canal obturation, compare with Tubli-seal (Zinc-Oxide base) and Sealepax (Calcium Hydroxyde base) sealers
Forty five single rooted human anterior teeth were instrumented and randomly divided into three experimental groups of 15 teeth each. All teeth in the experimental groups were obturated with laterally condensed gutta percha technique. Teeth in the first group were sealed using Zinc-Oxide (ZnO) based sealer and those of second group using Calcium Hydroxide (CaOH) based root canal sealer. Third experimental group was sealed using HA from School of Engineering USM. Teeth were then suspended in 2% methylene blue. After this, teeth were demineralized dehydrated and cleared. Linear dye penetration was determined under magnifying lense with calibrated eye piece.Statistical analyses of the linear dye penetration were performed with Kruskal Wallis test. As for the inter group comparison between HA and ZnO groups and between HA and CaOH groups were analysed by Mann-Whitney test. The dye penetration for group which were sealed with HA exhibited the lowest penetration and it showed that there was a statistically significant difference both between HA and ZnO groups and also between HA and CaOH groups ($p<0.001$).In conclusion, it was found that value added HA based endodontic material which were produced by USM can be used as a root canal sealing materials when it used in combination with epoxy resin since it leaked comparatively less as compared to ZnO and CaOH sealers. Before reaching a definitive conclusion, this material requires further extensive exploration both clinically and *in vitro*.

Key words: Apical seal, Endodontic sealers, Obturant, Methylsalicylate, Micro leakage

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INTRODUCTION

Root canal obturation consists of placing an inert filling material in the space previously occupied by pulp tissue. To achieve successful endodontic therapy, it is important to obturate the root canal system completely.

Gutta-percha is used with various techniques for obturation of the root canal system. Throughout the years, a variety of techniques using gutta-percha have been developed for root canal fillings. These techniques include lateral condensation, Kloroperka, Chloropercha, warm vertical condensation, injectable thermoplasticized, Ultrafill, and Thermofil. Investigators have evaluated the apical seals obtained by these various gutta-percha filling techniques.[1]

Lateral condensation remains the most widely accepted and used obturation technique.[2] As a result, all other techniques are compared to it to evaluate success. This study will also use lateral condensation for root canal obturation. In root canal obturation various materials have been proposed. The most frequent material used is gutta percha in combined with a root canal sealer. A root canal sealer must have ideal properties to be used for root canal obturation. Biocompatibility and sealing ability are fundamental to promote apical and periapical tissue repair.[3] Many materials are used for root canal sealer, but none of the available sealer consistency prevents leakage.[4] The hermetic sealing of the root canal space is one of the objectives in root canal therapy.[5]

The most common cause of failure involving endodontic therapy can be attributed to the lack of an apical seal leading to leakage at the apex. Effective endodontic obturation thus, must provide a dimensionally stable,inert fluid tight apical seal that will eliminate any portal of communication between the canal space and the surrounding periapical tissues through the apical foramen.[6]

Hydroxyapatite is the most thermodynamically stable synthetic calcium phosphate ceramic.[7] A calcium phosphate cement has indicated that is useful as a sealer because can seal a furcation perforation, is shown to be biocompatible and also has potential to promote the healing of bone in endodontic treatment.[8] Recently, most of the sealers commonly used contains zinc oxide or calcium hydroxide as a base ingredient of the powder.[9]

The present study was thereby designed to determine the sealing ability of hydroxyapatite when used as a sealer in root canal obturation, compare with Tubli-seal (zinc-oxide base) and Sealapex (calcium hydroxide base). Therefore, it would be interesting to examine whether or not hydroxyapatite is able to act as a root canal sealer. The rationale is that if adequate sealing is obtained, this material has the potential to be clinically useful.

MATERIALS AND METHOD

This study was designed to evaluate the *in vitro* sealing abilities of endodontic materials. The following materials were selected and grouping for the study.

A)Tubli_Seal ™ sealer (SybronEndo, Kerr/USA) a zinc-oxide based root canal sealer

B)Sealapex ™ sealer (SybronEndo, Kerr/USA) a calcium hydroxide based root canal sealer

C)Hydroxyapatite (HA) (from School of Engineering, Universiti Sains Malaysia) and were mixed with epoxy resin (Dentsply, DeTrey). This type of HA were used as part of a larger study (not yet published) in many fields and clinical trials.

The study was carried out *in vitro* on forty-five extracted human single rooted, noncarious anterior teeth which were collected from the outpatient department of oral and maxillofacial surgery, School of Dental Sciences, Universiti Sains Malaysia. All external debris was removed with an ultrasonic scaler.

All teeth randomly divided into 3 groups each is 15 teeth, group A, group B and group C sealers (as mentioned above). The crown were separated from the root until the length of the roots were 14 mm and store in saline. The pulps were broch and the root canals were prepared by Step-back technique with working length 13 mm until no:80 file with Master Apical File (MAF) no 50. After the use of each instrument (file), the root canals were irrigated with 1ml H2O2 3% and 1 ml NaOCl 2.5% and dry with paper point and ready for root canal filling. The sealer were mixed according the manufacture's directions. Hydroxyapatite granules were mixed up with epoxy resin liquid for hardener. The sealer was put along the lentulo plugger and coated to the inner walls of the canal by moving lentulo plugger clockwise according the groups, group A, group B and group C. One third apical of gutta percha master cone (no.50) was coated with sealer and seat in the canal to the full working length. The canal were obturated with lateral condensation technique. A finger spreader was inserted into the root canal to a level that was – 1mm short of the working length. The root canal was filled with accessory cones until the entire canal was obturated. The access cavities of teeth in all groups were then filled with Poly-F cement and Caviton, and then the root were immersed in saline solution for 4 weeks at 37°C. After storage, the roots were double coated with nail polish, with the exception of the apical 2 mm. Specimens used for the dye leakage test were placed in 2 % methylene blue solution (37 C, pH &) for 48 hours. The roots were then taken from the dye solution, remove the nail polish with le crown mesh, washed and dried with compressed air. The depth of dye penetration were evaluated with clearing method.[10]

All the teeth were immersed in HNO3 5% for 72 hours for teeth demineralized. HNO3 was changed with the fresh one every 24 hours. The teeth were then placed in alcohol 96% for 48 hours for teeth dehydration, and every 24 hours alcohol was changed with the fresh one. The final stage was clearing.[10] All the teeth were placed in methyl salicylate, until dye penetration were able measured visually. Apical leakage was measured from the apex to the most coronal extent of dye penetration (fig1, fig 2 and fig 3). Linear dye penetration was measured under magnifying lense with calibrated eye piece and analyse by Kruskal Wallis test. The intergroup comparison between hydroxyapatite and zinc oxide groups and between hydroxyapatite and calcium hydroxide groups were analysed by Mann-Whitney test. $P<0.05$ was considered statistically significant. SPSS 12.01 for windows [11],was used for data analysis.

RESULTS

Table 1. Amount of dye penetration in group A, B and C

Specimen No of each group	Amount of dye penetration (in mm)		
	Group A Tubli Seal (zinc oxide base)	Group B Sealapex (calcium hydroxide)	Group C (Hydroxyapatite)
1	2	2	0
2	1.5	1	0
3	2.5	1.5	0
4	2	2.5	1.0
5	2	3	0
6	1	1.5	0
7	2	3	0
8	3	2	0
9	1.5	3	0
10	2	2	0.5
11	3	2	0.5
12	2	3	0
13	1	3	0.5
14	2	2	0
15	2	3	0.3
Min - Max	1-3	1-3	0-1
Median (IQR)	2.0 (0.5)	2.0 (1.0)	0.0(0.5)

Table 2. Comparison of dye penetration (in mm) between study groups

Group	*n*	Dye Penetration		X^2 Stat. (*df*)	*P* value[a]
		Median (IQR)	Min.-Max.		
Zinc Oxide	15	2.0 (0.5)	1-3	31.00 (2)	<0.001
Calcium Hydroxide	15	2.0 (1.0)	1-3		
Hydroxyapatite	15	0.0 (0.5)	0-1		

[a] Kruskal Wallis test
IQR=Interquartile range; Min.=Minimum; Max.=Maximum;

Table 3 Comparison of dye penetration between Hydroxyapatite (HA) and others

Comparison	*Z* Stat.	*P* value[a]
Zinc Oxide vs HA	-4.76	<0.001
Calcium Hydroxide vs HA	-4.77	<0.001

[a] Mann-Whitney test; *P* value is adjusted using Bonferroni procedure for multiple comparison

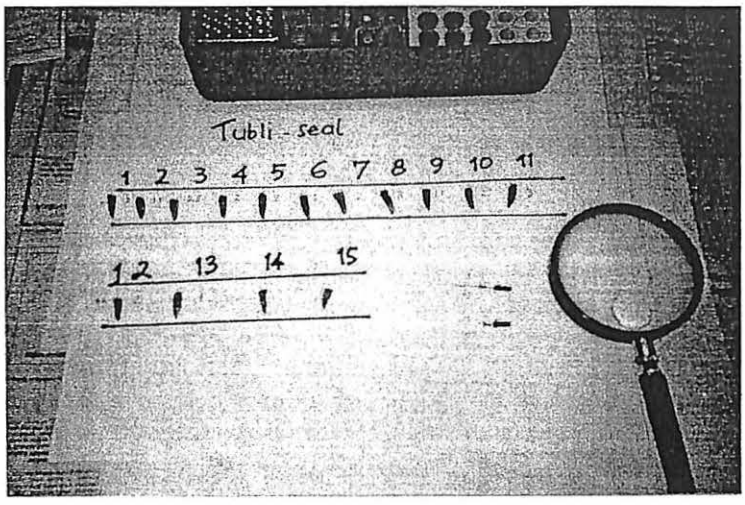

Fig1.Measurement of apical leakage in Tubli seal (ZnO base)

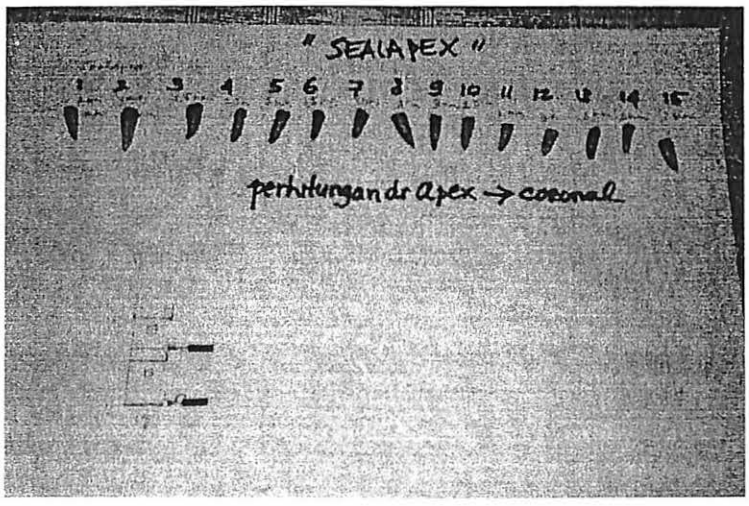

Fig2. Measurement of apical leakage in Sealapex (CaOH base)

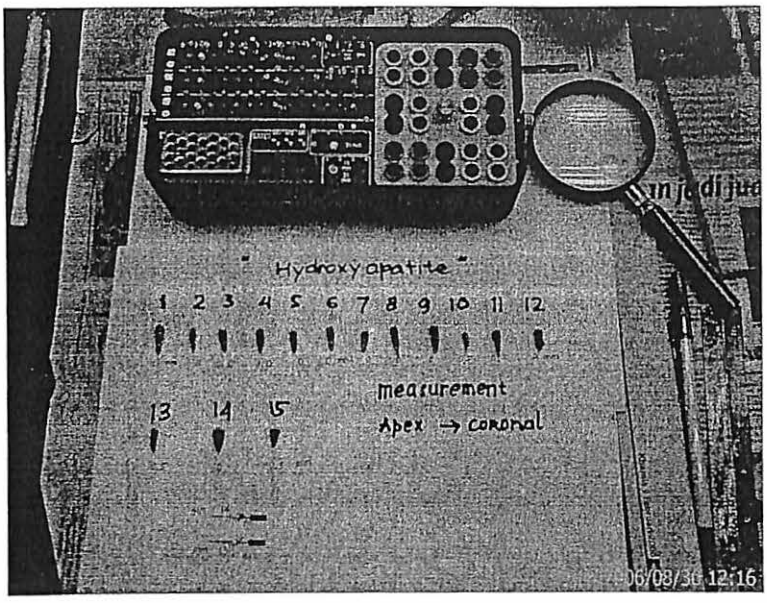

Fig3. Measurement of apical leakage in Hydroxyapatite

In the present study, measurements of maximum linear dye penetration were made to quantify the relative leakage (Figs 1,2,3). Dye penetration data for all the three groups are summarized in Table 1.

In group C teeth which were filled with laterally-condensed gutta-percha and hydroxyapatite (HA) sealer exhibited the lowest minimum-maximum (min-max) value of dye penetration. The min-max of dye penetration for group C(HA) was between 0-1mm. The min-max of dye penetration for group A teeth which were filled with laterally-condensed gutta-percha and zinc oxide (ZnO) base sealer was between 1-3 mm. The corresponding values for group B teeth which were filled with laterally condensed gutta-percha and calcium hydroxide (CaOH) base sealer was also between 1-3 mm. Ten samples in group C (HA) showed no dye penetration whereas all samples in group A and B showed dye penetration. The comparison of the three study groups using Kruskal Wallis test (table 2) revealed that there were at least one significant difference among the study groups ($p<0.001$).
Further Mann-Whitney test (post-hoe multiple comparisons between two groups) (table3) revealed that HA group has significancy lesser penetration compared to ZnO or CaOH groups ($p<0.001$)

DISCUSSION

HA is one of the ceramic materials commercially used for orthopaedic and dental implants, and it forms the principal mineral component of bone and comprises 60 % to 70 % of the calcified skeleton. It's chemical composition is Ca 10(PO4)6 (OH)2 and it has been produced synthetically since the early 1970's and used clinically for the last 20 years.[12,13] It has received considerable attention over the past two decades primarily because of it's excellent biocompatibility with hard tissues.[14,15] and when placed in contact with viable bone, result in osteoconduction and osteointegration.[16,17] HA does not cause a chronic inflammatory respons, toxic reactions or a foreign body giant cell reaction.[13] Although HA is a promising implant material, the greatest stumbling block to it's wider application and utilization is the brittleness of the material and it's

low strength for load-bearing applications.[18] Thus the material used in this study is the value added HA based material which were produced by a group of scientist at the School of Engineering Universiti Sains Malaysia (USM). The pure HA has been added with zirconia and other additional components, hot pressed and then sintered at a temperature of 1300^{0}C, to increase the toughness and strength of HA ceramic. Composites formed by HA ceramic in combination with zirconia have been proven not to produce any local or systemic adverse reactions or any cytotoxix effects in various *in vivo* studies.[19] This material showed no decrease in strength after ageing up to 1 year, which is in agreement with the study done by Shimizu et.al in 1993.[20]

It has been recognized for decades that the ideal end result of root canal therapy would be a closure of the apical foramen with reparative cementum. The goals for stability of successful endodontic therapy are total obliteration of the canal and perfect sealing of the apical foramen at the dentino-cemental junction and accessory canals at locations other than the root apex with an inert, dimensionally stable and biologically compatible material.[21] According to Timpawat et.al,[22] endodontic sealers are used to eliminate the interface between the gutta-percha and the dentinal walls. Thus, the quality of the filling depends largely on the sealing capacity offered by sealers.[23,24]

From this study an average leakage values of Zn O base sealer and CaOH base sealer both were minimum of 1 mm to maximum of 3 mm. The lesser value of dye penetration shown by value added HA sealer in the present study may be because of the better sealing abilities of HA. One possible explanation for this observed difference may be that HA has ability to bind strongly with natural bone tissue[25],and synthetic HA has the same chemical composition as biological HA and thus mimics many properties of natural bone.[26]As for epoxy-resin based endodontic sealers to the human dentin showed a higher capacity to attach to the dentinal walls than other endodontic sealers and provide bonding between it and gutta-percha points.[27]However the exact mechanism by which HA is incorporation with epoxy resin, then can be function as a good root canal sealer in the present study remain far from clear. It would be necessary to carry out further studies in order to make a larger evaluation of these value added HA based endodontic materials as well as their potential benefits. The *in vivo* evaluation should be done to assess the reaction to this value added HA as compared to the pure HA.

It can be concluded from this study that the value added HA based endodontic material which were produced by School of Engineering USM can be used as a root canal sealing materials when it used in combination with epoxy resin since it leaked comparatively less as compared to Zn O and CaOH sealers. Before reaching a definitive conclusion this material requires further extensive exploration both clinically and *in vitro*.

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